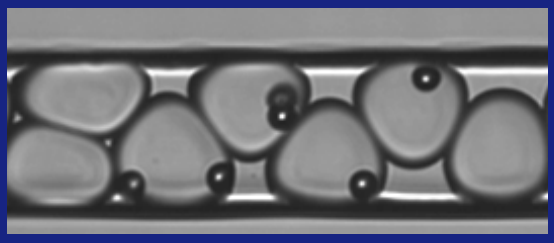
Sphere Fluidics

Cyto-Mine®

Cyto-Mine® Pooled Picodroplet Dispensing Protocol



Protocol version 1.0

This procedure should be used in conjunction with **SF-001907-UG** *Instrument Control – Cyto-Mine® Studio Suite* user manual.

Pooled picodroplet dispensing enables users to dispense all of the picodroplets sorted during an experiment into a single well of a 24-well microtiter plate.

Required Materials (not included)

Reagents

Product name	Supplier	Product code
OptiPrep™ Density Gradient Medium	Sigma-Aldrich Merck	D1556
Cyto-Surf® A	Sphere Fluidics	C303
Pico-Break™ 1	Sphere Fluidics	C081
Preferred culture medium (fresh)		

Equipment

- Cyto-Mine®
- Centrifuge
- 2 mm Hex Key
- 2.5 mm Hex Key
- 24-well microtiter plate
- 5 mL tube (sterile)
- 200 µL pipette
- 15 mL centrifuge tube

Cyto-Mine® Preparation

Before beginning an experiment, from the Main Menu use the Plate Operation button 

to eject the microtiter plate stage to enable configuration for a 24-well microtiter plate by removing the clamp plate spacer.

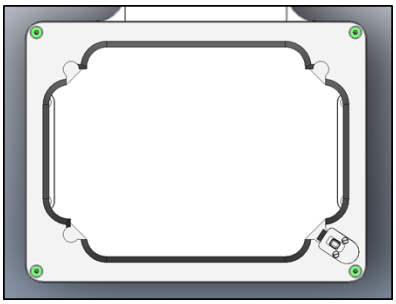
PLATE SPACER INSTALLED

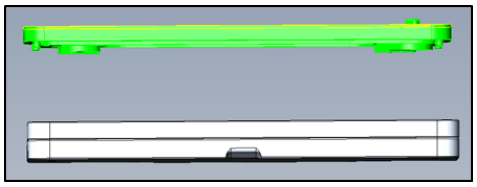
PLATE SPACER REMOVED

Plate stage modification

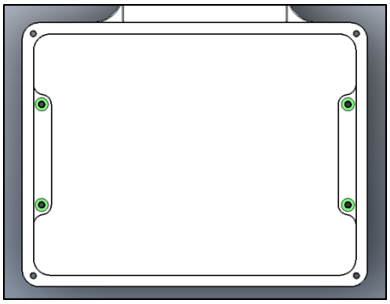
To remove the clamp plate spacer:

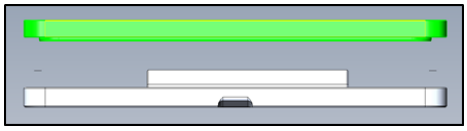
1. Eject the microtiter plate stage
2. Using a 2 mm Hex Key, unscrew the four securing screws and remove the well plate holder



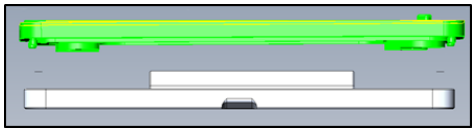


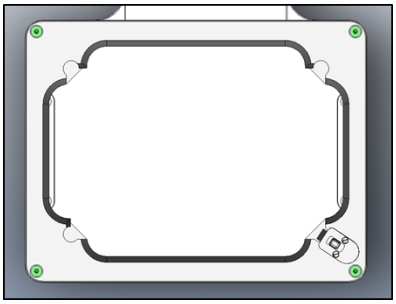
3. Using a 2.5 mm Hex Key, unscrew the four securing screws and remove the clamp plate spacer. Keep it in a safe place





4. Refit the well plate holder using the four plate holder securing screws





Pooled Picodroplet Dispensing

Prepare the 24-well Microtiter Plate

1. Into a 24-well microtiter plate, fill the first well (A1) with 1 mL fresh culture medium containing 16% (v/v) OptiPrep™ without serum
2. Carefully pipette a layer of 0.5 mL Cyto-Surf® A under the media in well A1

Load the 24-well Microtiter Plate

1. When prompted by the software, eject the plate stage, load the prepared 24-well microtiter plate and complete the pooled picodroplet dispensing (refer to 'Pooled Picodroplet Dispensing' in the Instrument Control - Cyto-Mine® Studio Suite Software v2.2 User Manual)
2. At the end of the experiment the plate stage will be ejected allowing you to retrieve the 24-well microtiter plate

Cell Recovery After Dispensing

1. Using a pipette, transfer the entire contents of well A1 to a sterile 5 mL tube (or other suitable vessel)
2. Keep the tube upright and stationary for about 1 minute, to ensure that the emulsion is floating on top
3. Carefully remove as much of the oil phase (lower phase) as possible without aspirating any of the aqueous phase/unbroken emulsion

Quick Tips!

- *For ease of extraction, use a thin, gel-loading pipette tip, alternatively a standard plastic pipette tip will suffice. This will minimize the amount of Pico-Break™ 1 that you will need to use to break the emulsion*
- *Do not fully depress the pipette before entering the oil. Once the pipette tip is in*

4. Add 1 mL Pico-Break™ 1 to the emulsion, close the lid of the tube and gently mix by inverting several times. You should see the emulsion start to disperse
5. Keep the tube upright and allow the mixture to settle for 2 minutes until de-emulsification is complete and the two phases are clearly separated. If de-emulsification is not complete after 2 minutes, repeat process by removing the bottom (Pico-Break™ 1) layer and adding an equal amount of fresh Pico-Break™ 1
6. After de-emulsification, the bottom layer is the unwanted fluorous phase. The top phase contains your sample of interest
7. Using a 200 µL pipette, carefully remove the top aqueous layer and transfer to a clean 15 mL centrifuge tube

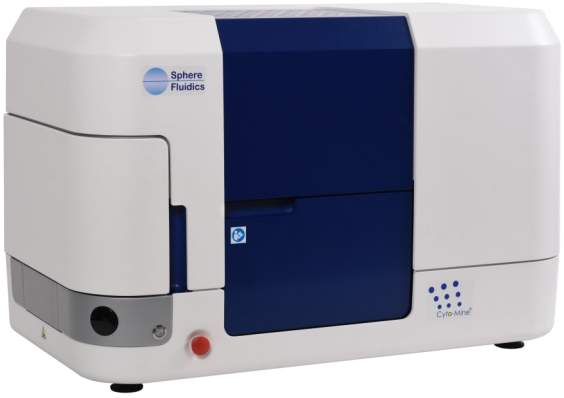
Quick Tips!

- *Make sure the sample is kept still for 1-2 minutes after de-emulsification, before recovering the aqueous phase, this will allow the oil drops in the aqueous phase to sediment and minimize carrying over oil*
- *We recommend carrying out multiple removal steps with a 200 uL pipette to remove the aqueous phase and leave 100 µL of the top layer behind to prevent any transfer of oil. To recover any remaining cells, add 900 µL fresh culture medium to the aqueous phase, let stand briefly for phases to separate and remove the aqueous phase, again making sure not to aspirate any oil. If desired,*

8. After recovering the aqueous phase, dilute at least five-fold by adding fresh medium without OptiPrep™ (Note: OptiPrep™ is a density gradient medium and will interfere with cell sedimentation during centrifugation unless it is sufficiently diluted)
9. Centrifuge at 300 *x g* for 5 - 10 minutes
10. Remove the supernatant without disrupting the cell pellet
11. Resuspend cells in an appropriate volume of culture medium
12. Culture the cells as per your standard protocol

Cyto-Mine®

The Single Cell Analysis and Monoclonality Assurance System



Code	Product Ordering Information
S003	Cyto-Mine® System
C301	Cyto-Mine® Consumables Suite
C302	Cyto-Cartridge® Pack of 5
C303	Cyto-Surf® A (250ml)
C304	Cyto-Surf® B (250ml)
C310	Cyto-Cellect™ Human IgGκ Detection kit
C081	Pico-Break™ 1

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All Cyto-Mine® components, the Cyto-Cartridge® and Sphere Fluidics' supplied chemicals and bioreagents are Animal Origin Free and GLP-compliant.

For research use only.
Product specifications subject to change without notice.

CE

ISO 9001

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